A Case-Control Study of Nitrate in Drinking Water and Non-Hodgkin's Lymphoma in Minnesota

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ABSTRACT. Nitrate in drinking water has been implicated as a possible risk factor for non-Hodgkin's lymphoma. The authors examined the association between non-Hodgkin's lymphoma and waterborne nitrate through a population-based case-control study of white men in Minnesota. The authors, by linking residential histories with community water records, estimated average long-term exposure to nitrate in drinking water from 1947 to 1975 for 73 cases diagnosed between 1980 and 1982 and for 147 controls who used community water supplies. No association was found between nitrate levels in community water supplies and non-Hodgkin's lymphoma within the range of study exposures (median of highest exposure category = 2.4 mg nitrate/l [range = 0.1–7.2 mg/l]). The findings provide some safety assurance for those who use water systems that have nitrate levels that are less than 2.4 mg/l.

IN THE UNITED STATES, the increase in non-Hodgkin's lymphoma (NHL) mortality between 1950 and 1980 occurred disproportionately in the agricultural midwest.[1] In several epidemiologic studies, researchers examined NHL risk from pesticide exposures in this geographic area,[2–7] but excess risk remains substantially unexplained.[8]

The increase in fertilizer use[9] during the past 40 y has contributed to nitrate contamination of drinking water in rural areas. Some individuals have hypothesized that nitrate may be a risk factor for cancer through its role as a precursor compound in the formation of *N*-nitroso compounds, many of which are animal carcinogens.[10] The results of a recent population-based case-control study in Nebraska suggested that long-term consumption of nitrate in drinking water has contributed to NHL risk.[11]

We further explored the association between nitrate in drinking water and NHL by evaluating the risk of NHL from average long-term consumption of nitrate in drinking water in Minnesota—a state with heavy nitrogen fertilizer use.[12]

Method

Study framework. The data collected in the present study supplement the data collected in the 1980 study in which in-person interviews of 329 incident cases of NHL and 642 population-based controls were conducted. The design and data collection in the original study,

which also included cases of leukemia, are described in detail elsewhere.[2]

Briefly, in the original study, the investigators ascertained all cases of NHL among white men aged 30 y or older who were diagnosed between 1980 and 1982 from Minnesota hospital and pathology laboratory records. Eligibility was limited to cases who resided in areas of Minnesota other than the four largest cities. Four experienced pathologists reviewed all NHL diagnoses and classified them according to the working formulation for NHL.[13] We selected controls randomly from white men who lived in Minnesota and frequency-matched them to NHL and leukemia cases by age in 5-y groups and by vital status at the time of interview.

We conducted in-person interviews with a structured questionnaire. The questionnaire included information about sociodemographic, medical, behavioral, and occupational characteristics, as well as questions about consumption of beverages and dairy products. The questionnaire also contained references to lifetime residential history and historical sources of drinking water. We then asked the participants to provide the location and period of each residence at which they had lived for at least 1 y, as well as what served as their primary source of drinking water at each residence (i.e., community supply, private well, bottled water, and other source).

Study population. Given that historic nitrate levels in private wells are unknown, we restricted our study to cases and controls who had reported using Minnesota community water supplies or bottled water for at least 90% of the years between 1947 and 1980. A total of 73 cases and 147 controls who used water from 157 communities in Minnesota met this criterion. To check our analysis, we also assessed risk with alternative inclusion criteria for the study population, based on varying percentages of years (i.e., 60%–100%) that community water supplies were used in Minnesota.

Classification of exposure to waterborne nitrates. We obtained records on laboratory nitrate measurements (on the basis of nitrogen in nitrate) in Minnesota community water supplies from the Minnesota Department of Health (MDH) for the period between 1947 and 1984. We used nitrate measurements from both public wells and distribution points (e.g., schools) to calculate annual nitrate levels in the drinking water of each community between 1947 and 1980. For any nondetectable nitrate value, we assigned a value of one-half the detection threshold to limit misclassification.

To fill in years for which data were missing and to use all information in the estimation of exposure in years with data, we calculated a time-weighted nitrate average for each year in each community. Measurements within 2 y of the year being estimated were weighted as 1; ≥ 2–5 y, as 0.5; and > 5–10 y, as 0.2. We weighted measurements that exceeded 10 y as 0.01 to ensure an estimate for early years (i.e., when some towns had no records). We accorded additional weight to distribution-point measurements, which reflected actual tapwater delivered to community residents.

We linked participants' reported residential histories with estimated nitrate level by year in each community of residence. For any year during which participants reported the use of bottled water, we assumed the nitrate level was 0. We then calculated an estimated arithmetic average nitrate exposure for cases and controls for the period 1947 through 1975 to allow a 5-y lag period from 1980—the earliest date of diagnosis. We also calculated exposure on the basis of an unlagged period.

We set the exposure referent at ≤ 0.5 mg/l—half the detection limit prior to about 1975. The remaining average values were grouped into tertiles, with the highest category set at > 1.5 mg/l.

To provide a further check on our analysis, we used other algorithms, with different weights applied to nitrate measurements, to estimate average exposure. We also examined the association with maximum nitrate exposure for the years 1947–1975. In setting the maximum exposure reference value at ≤ 0.5 mg/l, we grouped the remaining values by tertiles, with the highest category set at > 1.9 mg/l.

Statistical methods. We used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals (CIs).[14] To examine potential confounders, we used questionnaire information about age (as a categorical variable), marital status, education, cancer history, occupational exposure, smoking and drinking histories, farming history, use of hair dye, pesticide use, and proximity to industrial sites. Inasmuch as adjusting for potential confounders did not affect the crude OR appreciably, only age-adjusted ORs and 95% CIs are presented herein.

Results

Characteristics identified in the questionnaire were compared with respect to cases and controls (Table 1). Among other things, cases were more likely than controls to be younger, to have a family history of cancer, and to have an occupation associated previously with NHL.

We found no association between NHL and long-term average nitrate exposure from community water supplies within the exposure range of this study (i.e., 0.1–7.2 mg/l [Table 2]). The age-adjusted association (i.e., OR) was 0.3 in the highest category (i.e., > 1.5 mg/l), with a median nitrate average of 2.4 mg/l.

Results were similar for the alternative-exposure algorithms tested. No association with drinking water nitrate was found when we unlagged the exposure variable. When we modified the inclusion criteria (based on percentage of years community water supplies were used), we found no association between NHL and average waterborne nitrate. When direct and surrogate responders were analyzed separately, we again failed to observe an association. Finally, NHL was not associated with maximum nitrate exposure.

Discussion

Our study had several advantages over many previous studies in which cancer risk versus nitrate in drink-

Table 1.—Characteristics of Cases and Controls in the Community Water Analysis*

Characteristic	Cases (*n* = 73) No.	%	Controls (*n* = 147) No.	%
Age (y)				
< 60	22	30	24	16
60–69	15	21	47	32
70+	36	49	76	52
Family history of cancer				
No	31	42	69	47
Yes	41	56	67	46
Ever worked on farm as adult				
No	59	81	89	61
Yes	14	19	58	39
Ever had high-risk occupation†				
No	61	84	130	88
Yes	12	16	16	11
Education (y)				
≤ 12 (or vocational)	47	64	116	79
> 12	26	36	31	21
Ever used hair coloring				
No	70	96	140	95
Yes	3	4	5	3
Ever used tobacco daily				
No	9	12	30	20
Yes	64	88	117	80
Ever used insecticides monthly				
No	62	85	136	93
Yes	9	12	9	6
Ever used herbicides monthly				
No	68	93	141	96
Yes	3	4	4	3
Ever lived .5 mi from factory				
No	49	67	93	63
Yes	24	33	54	37

*Study participants = individuals who spent at least 90% of their lives between the years 1947 and 1980 consuming community water supplies in Minnesota or bottled water anywhere.
†Occupations identified in published literature as associated with non-Hodgkin's lymphoma, including machinists, photoengravers, cosmetologists, embalmers, meat workers, rubber workers, foundry workers, pulp and paper mill workers, leather workers, aircraft and parts workers, and fabricated metal industry workers.

Table 2.—Age-Adjusted Odds Ratios (ORs) and 95% Confidence Intervals (CIs) for Non-Hodgkin's Lymphoma and Long-Term Average Nitrate Exposure from Community Water Supplies*

Average nitrate (mg/l)†	Cases (*n* = 73) No.	%	Controls (*n* = 147) No.	%	OR	95% CI
≤ .05	41	56	83	56	1.0	
> 0.5 to ≤ 1.5‡	29	40	44	30	1.4	0.7, 2.5
> 1.5	3	4	20	14	0.3	0.1, 0.9

*Study participants = individuals who spent at least 90% of their lives between the years 1947 and 1980 consuming community water supplies in Minnesota or bottled water anywhere.
†Average, based on period between the years 1947 and 1975.
‡Categories originally set at 0.5 to ≤ .07 and 0.7 to ≤ 1.5 were merged because the ORs were similar.

ing water has been studied. In our study, exposure classification was better than in previous studies, which have been ecologic or have been based on short-term measurements of nitrate[15–22] (i.e., designs more vulnerable to misclassification).

We obtained nitrate levels in community water supplies for more than 30 y and linked them with each participant's individual residential history. The method of inputing missing data was the same for cases and controls; therefore, the likelihood of differentially biased estimates was reduced. In only one other study[11] have investigators considered individual exposure histories, and they found a significant association between NHL and long-term average exposures ≥ 4 mg/l among community water supply users; such a level, however, exceeded the estimated exposure of most of the participants in our study and, therefore, does not conflict with our findings.

An additional advantage of our study was that the questionnaire provided information about many risk factors potentially applicable to our population, although none of the factors proved to be confounders with respect to waterborne nitrate. Also, whereas we did not include other waterborne contaminants in our model, other contaminants have not been linked strongly to NHL.[23] Moreover, given our negative finding, had we controlled for other factors that might have been associated with both NHL and nitrate, we likely would not have found a positive finding in our study.

Our findings are also supported by several alternative analyses of our data, which yielded similar results to our initial analysis. There was no association with NHL, regardless of (a) the algorithm selected for the estimation of exposure, (b) whether direct responders or surrogate responders were analyzed, or (c) whether other inclusion criteria were used. Risk of NHL was also not associated with long-term maximum nitrate exposure.

Nonetheless, limitations in our ability to estimate nitrate exposure did exist. Sources of potential exposure misclassification included the absence of information about the amount of water consumed, the contribution of different community wells to the drinking water distributed, and the nitrate intake from drinking water at the worksite. Another potential source of misclassification was the sparseness of community nitrate measurements in much earlier years. Given that the resulting misclassification presumably did not differentiate between cases and controls, it is unlikely that our findings masked a "true" positive association.

The results of government surveys indicate that of the 219 million Americans who use community-water supplies, approximately 92 million ingest drinking water with nitrate levels below 0.3 mg/l.[24] The results of our study suggest that concentrations of nitrate in water of 2.4 mg/l do not pose a risk of NHL and, therefore, provide some assurance of safety to many Americans.

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